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ASSEMBLY OF PROTEORHODOPSIN FOR BIOINSPIRED ALTERNATIVE ENERGY APPLICATIONS

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ABSTRACT

Proteorhodopsin is an integral membrane protein that functions as a light-driven proton pump, which serves as the basis for an alternative energy device. While many membrane proteins are becoming understood, both structurally and mechanistically, little technology exists for the production and incorporation of this important class of molecules into useful devices. The advantage we have in working with proteorhodopsin is that it can be genetically modified and grown in the laboratory to express different optical and charge properties. The strategy to functionally assemble proteorhodopsin requires manipulation of its charge interaction for induced self-assembly in a lipid bilayer, followed by the binding of the protein-lipid complex onto a conductive substrate, such as Kapton™ or borosilicate coated with indium tin oxide.

1. INTRODUCTION

In 2000, a new family of photoactive trans-membrane protein, known as proteorhodopsin, was identified through genomic analysis of marine bacterioplankton (Beja et al., 2000). Subsequent reports show that proteorhodopsin is ubiquitous in marine environments (Beja et al., 2001). The interest in proteorhodopsin is because of its demonstrated ability to pump protons across cell membranes (Beja et al., 2000). In nature, this action produces a pH gradient that the organism uses to generate ATP for cellular energy. In the laboratory, variants can be easily expressed in *E. coli* to obtain spectrally tuned protein with variances in pH stability and proton pumping efficiency.

The ability to manufacture proton-tight, artificial, biological membranes containing functional, oriented proteins is the limiting technology for a variety of devices ranging from biosensors to photovoltaic cells. While many membrane proteins, such as receptors, transporters, light driven proton pumps, and catalysts are becoming understood, both structurally and mechanistically, little technology exists for the production and incorporation of this important class of molecules into useful devices. In this work, we are developing a cooperative assembly process that will be used as the basis for functional proteo-gradient membrane units.

2. MATERIALS AND METHODS

Proteorhodopsin was expressed and purified as reported (Kelemen et al., 2003). Liposome membranes consisting of 1,2-dioleoyl-3-trimethylammonium-propane (chloride salt) and 1,2-dioleoyl-sn-glycero-3-phosphocholine were purchased from Avanti Polar Lipids, Alabaster, AL, and prepared as reported (Liang et al., 2004). N-dodecyl-ß-D-maltoside, purchased from Sigma-Aldrich, was used at a concentration of 0.05% in buffer solutions to maintain proteorhodopsin in its solubilized form.

3. RESULTS

We have achieved a comprehensive understanding of the size and photoactive properties of the proteorhodopsin and have assembled the protein into a functional two-dimensional and three-dimensional membrane configuration. Preliminary results show that proteorhodopsin can cooperatively assemble with artificial biological lipid membranes to form multi-lamellar structures. Depending on the pH, membrane charge density and hydrophilic loop region sequences, the protein will organize into different arrays of two-dimensional lattices. Furthermore, adjacent membranes can couple to form multi-lamellar superlattices (Liang et al., 2007, 2008). The work with Kapton™ and borosilicate coated indium tin oxide are being further developed.

CONCLUSION

Our objective is to design and express proteorhodopsin variants of interest on a large scale, to assemble the protein into functional proteo-gradient membrane units, to hybridize them with conductive substrates that maintain the biological function of the protein and are mechanically stable. Achieving these objectives allows us to create a bio-inspired alternative energy device, such as a photovoltaic device or fuel cell that will convert clean and abundant solar energy into usable electric power. Such a resource allows warfighters to be more self-sufficient and less reliant on host country support, especially if the host nation infrastructure is minimal, or for operational security necessities.

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